

APPLICATIONS

A Rapid Extraction of Corticosteroids from Urine Using a Strata[®] RP On-Line Solid Phase Extraction Column

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Overview

In this application note, corticosteroids are extracted from urine using on-line extraction with a Thermo Cohesive System run in TX mode. A 50 x 0.5 mm Strata RP solid phase extraction (SPE) column in conjunction with a 50 x 3.0 mm Kinetex[®] 2.6 μm Biphenyl analytical LC column results in a total runtime of less than six minutes including system equilibration. **Figure 1** displays the Aria[®] based software overview and **Table 1** provides the on-line SPE conditions and **Table 2** shows the LC methodology. **Table 3** displays the calculated assay recovery, showing that all nine compounds except for Triamcinolone (the most polar compound in the suite) provide recovery greater than 73% and in all cases, show relative standard deviation less than 12.1%. **Figure 2** is a representative TIC for a 50 ng/mL spike into urine.

Materials

Standards were purchased from Cerilliant[®] (Round Rock, TX). Double charcoal stripped human serum was purchased from BioreclamationIVT[®] (Westbury, NY) All other reagents and chemicals were obtained from Sigma-Aldrich[®].

Experimental Conditions

Sample Pre-treatment:

Human urine is diluted 1:1 with 10 mM Ammonium acetate.

On-Line Solid Phase Extraction

On-line SPE Column: Strata RP
Dimensions: 50 x 0.5 mm
Part No.: 00B-S326-AF

LC-MS/MS Conditions

LC Column: Kinetex 2.6 μm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
Guard Column: SecurityGuard[™] ULTRA Cartridges
Part No.: AJ0-9208
Mobile Phase: A: 10 mM Ammonium Acetate in Water
B: 10 mM Ammonium Acetate in Methanol
Gradient: See Table 2
Flow Rate: See Table 2
Needle Wash 1: Methanol/Water (50:50)
Needle Wash 2: 0.1 % Formic acid in Water
Injection Volume: 30 μL
Instrument: Cohesive System run in TX Mode: Agilent[®] 1260 with Leap Technologies PAL[®] autosampler LX-2
Detector: MS/MS (SCIEX 4000 QTRAP[®]) ESI+ @ 400 °C

Figure 1.
Screenshot of Method from Aria Software

Extraction Column Conditions (blue) Analytical Column Conditions (Pink)

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	50.0	50.0
2	0.50	5	0.15	Step	30.0	70.0	-	-	====	in	0.25	Step	50.0	50.0
3	0.58	40	0.15	Step	10.0	90.0	-	-	T	in	0.25	Step	50.0	50.0
4	1.25	30	0.80	Step	10.0	90.0	-	-	====	in	0.40	Step	50.0	50.0
5	1.75	60	0.80	Step	-	100.0	-	-	====	in	0.40	Step	50.0	50.0
6	2.75	60	0.80	Step	-	100.0	-	-	====	out	0.40	Step	10.0	90.0
7	3.75	45	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	10.0	90.0
8	4.50	75	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	50.0	50.0
9	5.75	5	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	50.0	50.0

Flow Rate Unit in mL/min



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Table 1.
On-line SPE Conditions

Step	Time (min)	Flow Rate (mL/min)	0.1 % Formic acid in Water	0.1 % Formic acid in Methanol
1	0	0.8	70	30
2	0.5	0.15	30	70
3	0.58	0.15	10	90
4	1.25	0.8	10	90
5	1.75	0.8	0	100
6	2.75	0.8	0	100
7	3.75	0.8	70	30
8	4	0.8	70	30
9	5.75	0.8	70	30

Table 2.
LC Conditions

Step	Time (min)	Flow Rate (mL/min)	0.1 % Formic acid in Water	0.1 % Formic acid in Methanol
1	0	0.4	50	50
2	0.5	0.25	50	50
3	0.58	0.25	50	50
4	1.25	0.4	50	50
5	1.75	0.4	50	50
6	2.75	0.4	10	90
7	3.75	0.4	10	90
8	4.5	0.4	50	50
9	5.75	0.4	50	50

Table 3.
Assay Recovery Values

Analyte	% Recovery	% CV (n=6)
11- α -OH Progesterone	92.5	5.54
Triamcinolone Acetonide	93.7	12
Cortisone*	130	7.94
Betamethasone	73.4	4.82
Triamcinolone	50.3	8.25
Prednisone	84	4.28
Cortisone Acetate	91.4	10
Corticosterone	87.5	3.17
Testosterone	84	5.99

Assay Recovery = Analytes in Matrix / Analytes in Neat solution with TX mode

*Blank urine contains endogenous level of cortisone

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Figure 2.
Representative of a Chromatogram of Human Urine at 50 ng/mL

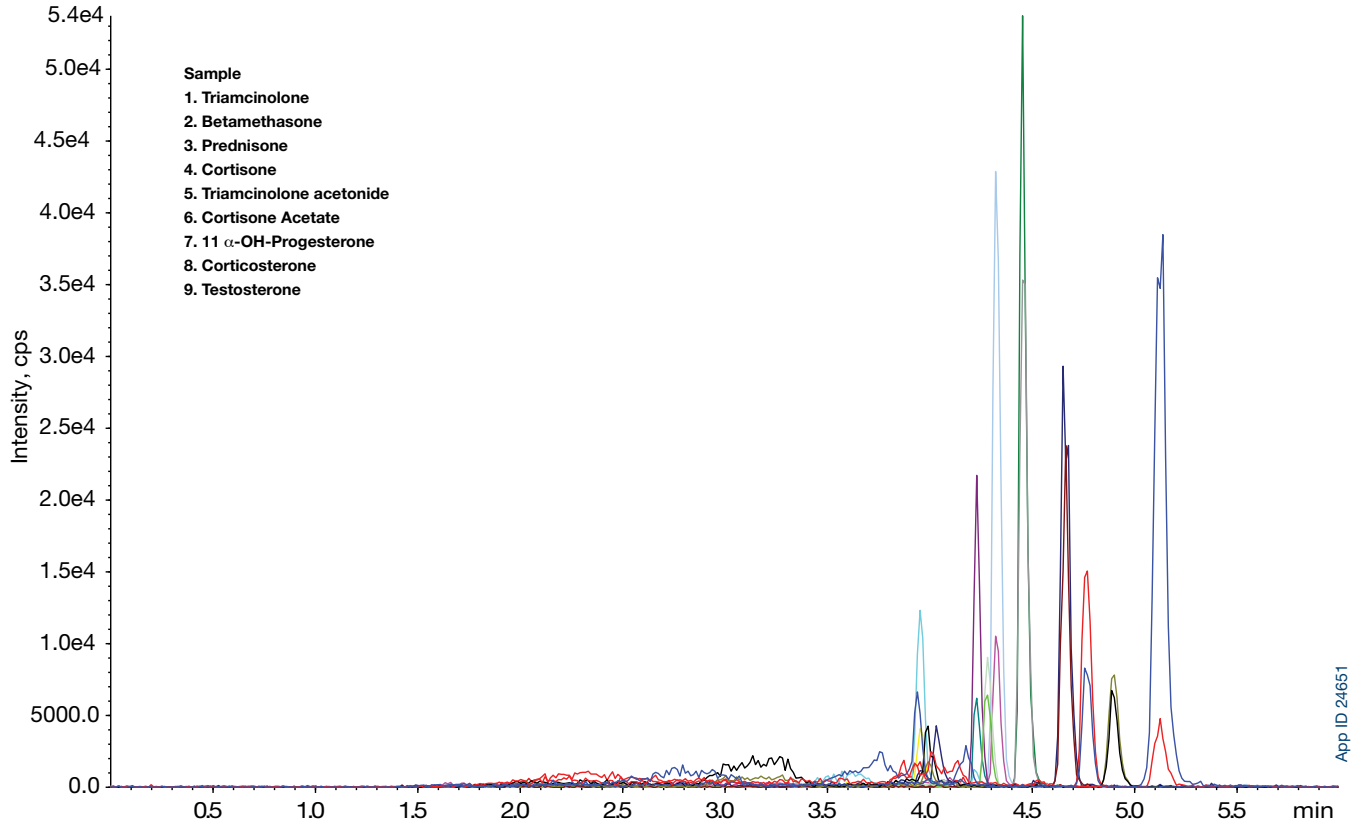
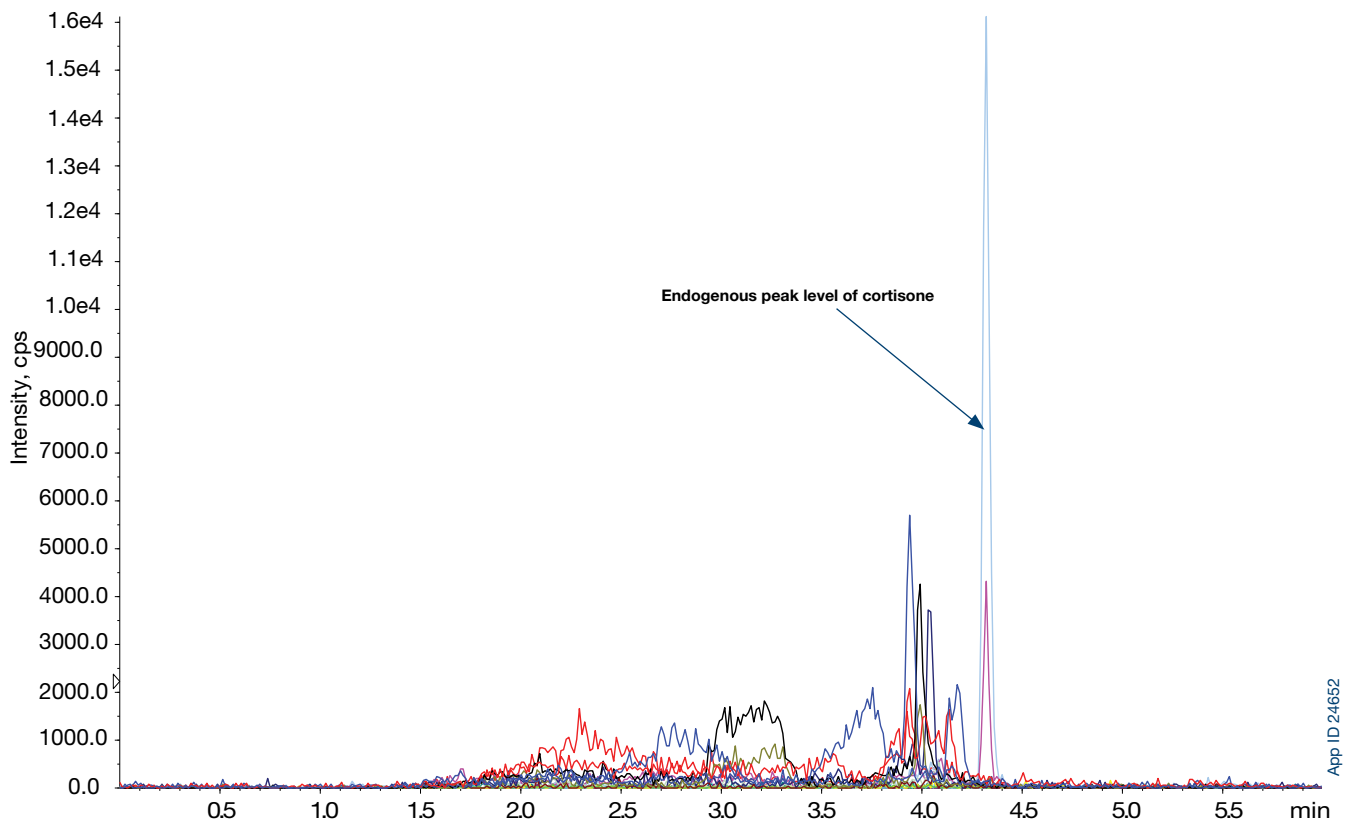


Figure 3.
Representative of a Chromatogram of Blank Human Urine



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Ordering Information Strata® On-Line SPE

On-Line Extraction Columns (mm)			
Phase	50 x 0.5	50 x 1.0	20 x 2.1
Strata RP	00B-S326-AF	00B-S326-AO	00M-S326-AN

Kinetex® Core Shell LC Columns

Kinetex 2.6 µm MidBore™ Columns (mm)			SecurityGuard™ ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	150 x 3.0
Biphenyl	00B-4622-YO	00D-4622-YO	00F-4622-YO
			AJ0-9208 for 3.0 mm ID

† SecurityGuard ULTRA Cartridges required holder, Part No.: AJ0-9000.

Other particle sizes and dimensions are available. Visit www.phenomenex.com/Kinetex for a complete list of available columns.

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